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Complementary Value of CEUS-Guided Hookwire Localization Combined with Methylene Blue Staining for Sentinel Lymph Node Detection, and the Predictive Role of Imaging Grade for Nodal Metastasis: A Single-Arm Clinical Study

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Research Article

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Abstract

Background

Accurate identification of sentinel lymph nodes (SLNs) is essential for tumor staging and surgical decision-making. Although dye-guided SLN biopsy is widely used in clinical practice, relying on a single detection modality may lead to imprecise localization or missed detection of SLNs. Contrast-enhanced ultrasound (CEUS) has recently emerged as a promising imaging technique for visualizing lymphatic drainage and identifying sentinel lymph nodes.

Objective

This study aimed to evaluate the complementary detection performance of CEUS-guided hookwire localization combined with methylene blue staining for sentinel lymph node identification and to explore the potential predictive value of CEUS imaging grade for SLN metastasis.

Methods

A total of 76 patients who underwent sentinel lymph node biopsy were included in this single-arm clinical study. All patients underwent preoperative CEUS-guided hookwire localization followed by intraoperative methylene blue staining for SLN identification. The detection patterns of SLNs by CEUS and methylene blue were recorded and compared. In addition, CEUS imaging classified lymph nodes into three grades (I, II, and III), and the association between CEUS grade and pathological SLN metastasis was analyzed.

Results

Sentinel lymph nodes were successfully identified in 73 of 76 patients, yielding an overall detection rate of 96.05%. A total of 79 SLNs were excised, with a mean of 1.04 nodes per patient. CEUS-guided hookwire localization and methylene blue staining demonstrated partial overlap in SLN identification. On a patient basis, 71 patients (93.42%) were identified by CEUS-guided hookwire localization, whereas 69 patients (90.79%) were identified by methylene blue staining, indicating complementary detection performance between the two techniques. Pathological examination revealed SLN metastasis in 7 patients (9.21%). Among these metastatic cases, SLNs were identified by either CEUS-guided hookwire localization or methylene blue staining in 7 patients, including 2 patients detected by CEUS-guided hookwire localization alone, 1 patient detected by methylene blue staining alone, and 4 patients detected by both methods. Furthermore, CEUS imaging grade was associated with SLN positivity. The positivity rates were 1.82% in grade I lymph nodes, 15.00% in grade II lymph nodes, and 100.00% in grade III lymph nodes. Conclusion: CEUS-guided hookwire localization combined with methylene blue staining demonstrates potential complementary value in sentinel lymph node detection. In addition, CEUS

imaging grade may provide useful information for predicting SLN metastasis, suggesting a potential role of CEUS in improving the precision of SLN identification and risk stratification.

1. Introduction

Sentinel lymph node biopsy (SLNB) is an established standard approach for regional nodal staging in patients with early-stage breast cancer, because it provides essential axillary staging information while reducing surgical morbidity compared with axillary lymph node dissection (ALND), including lymphedema and sensory impairment (1, 2). The sentinel lymph node (SLN), defined as the first lymph node receiving lymphatic drainage from the primary tumor, is considered representative of the regional nodal basin and therefore has important implications for staging, treatment decision-making, and prognosis (2, 3).

At present, several techniques are available for SLN mapping, including blue dye-guided methods, radiotracers, fluorescence imaging, and newer image-guided approaches such as contrast-enhanced ultrasound (CEUS) (3). Among them, methylene blue staining remains widely used in many centers because of its practicality and accessibility, particularly where nuclear medicine resources are limited(4–6). However, dye-guided approaches have recognized limitations. Blue dye cannot provide reliable pre-incision localization; its performance is affected by lymphatic drainage variability, and the identification process remains dependent on intraoperative visualization and surgical experience (4–6). These limitations may be particularly relevant in patients with complex lymphatic anatomy or deeper nodal locations, raising the possibility that reliance on a single tracer could lead to incomplete SLN identification.

CEUS has emerged as a promising imaging technique for SLN localization and characterization in breast cancer. After peritumoral or periareolar injection of microbubble contrast agents, CEUS can visualize lymphatic channels and SLNs in real time before surgery, thereby providing both anatomical and perfusion-related information (5, 6). Recent prospective studies have shown encouraging diagnostic performance. In a 2021 preliminary study, CEUS achieved a sensitivity of 82.67% and a specificity of 80.36% for SLN status assessment (7). In a 2023 prospective study, CEUS identified SLNs in 100% of examined patients, and enhancement patterns III/IV were associated with substantially higher metastatic risk (8). More recently, a 2025 prospective diagnostic study reported an SLN localization rate of 98.4%, with a sensitivity of 99.0% and a specificity of 88.1% when metastatic enhancement patterns were applied(2). In parallel, an updated meta-analysis published in 2023 confirmed the overall diagnostic robustness of CEUS for SLN metastasis in breast cancer, showing high pooled sensitivity and specificity(9).

Because methylene blue staining and CEUS are based on different detection principles, they may provide complementary information during SLN evaluation. Methylene blue primarily facilitates intraoperative visual tracing, whereas CEUS offers preoperative visualization of lymphatic drainage pathways and nodal enhancement characteristics [3,5]. Previous clinical studies have suggested that combining CEUS with

blue dye is feasible and may improve SLN detection performance compared with conventional dye-guided approaches alone(4, 10). In addition, CEUS enhancement patterns appear to correlate with pathological nodal status, suggesting that CEUS may serve not only as a localization tool but also as a potential method for preoperative risk stratification of SLN metastasis(2, 8, 9).

Nevertheless, evidence directly evaluating the complementary detection value of CEUS and methylene blue staining remains limited. Most published studies have focused either on CEUS-based localization and metastasis prediction or on conventional tracer-guided SLNB. In contrast, relatively few have systematically examined the overlap, discrepancy, and potential complementarity between CEUS and methylene blue in the same clinical cohort.

Therefore, the present study aimed to evaluate the clinical performance of CEUS-guided hookwire localization combined with methylene blue staining in patients undergoing SLNB. Specifically, we investigated the SLN detection patterns of both CEUS and methylene blue, assessed their overlap and complementarity, and explored the association between CEUS imaging grade and pathological SLN metastasis in order to determine the potential predictive utility of CEUS.

2. Methods

2.1 Study Design and Patients

This single-arm, prospective study was conducted at Jiangyou 903 Hospital between August 2023 and December 2025. A total of 76 patients diagnosed with early-stage breast cancer and scheduled for sentinel lymph node biopsy (SLNB) were enrolled.

Inclusion criteria:

· Age ≥ 18 and < 70 years

· Clinically and pathologically node-negative breast cancer

· No prior axillary surgery

Exclusion criteria:

· Pathologically confirmed axillary lymph node metastasis by preoperative biopsy

· Allergy to methylene blue or ultrasound contrast agents

· Pregnancy or lactation

· Severe comorbidities precluding surgery

All patients provided written informed consent, and the study protocol was approved by the institutional review board of Jiangyou 903 Hospital.

2.2 Contrast-Enhanced Ultrasound (CEUS) Procedure with Hookwire Localization

· Equipment: Resona 7 ultrasound system with a 12 MHz linear-array probe.

· Contrast agent: SonoVue, total dose: 0.4 mL (0.2 mL per injection site)

· Injection site and method: CEUS contrast agent was injected intradermally at two sites:

a. 12 o'clock position around the areola

b. Lateral aspect of the areola (left breast at 3 o'clock; right breast at 9 o'clock)

Procedure:

a. CEUS was performed preoperatively to visualize lymphatic channels and identify sentinel lymph nodes (SLNs).

b. SLNs were classified into three grades based on enhancement patterns:

o Grade I: homogeneous enhancement with clearly defined cortex and hilum

o Grade II: partial or heterogeneous enhancement

o Grade III: irregular or disrupted enhancement, suspected metastasis

c. Hookwire localization: After CEUS identified the target SLN(s), a sterile hookwire was inserted under real-time ultrasound guidance into the SLN. The position of the hookwire tip was confirmed within the node, and the wire was secured at the skin surface to guide intraoperative excision.

d. Successful localization was defined as clear visualization of at least one SLN and proper hookwire placement suitable for surgical excision.

2.3 Methylene Blue Staining and SLN Excision

· Injection site and method: Methylene blue was injected subcutaneously at the same two sites as CEUS — the 12 o'clock position around the areola and the lateral aspect of the areola (left breast at 3 o'clock; right breast at 9 o'clock). Dose: 0.5 mL per site.

· Injection timing: 10–15 minutes before skin incision

Surgical procedure:

1. Intraoperatively, the sentinel lymph nodes (SLNs) were localized by visual inspection of the blue dye and by manipulating the tail of the hookwire. Standard SLNB was performed under dual guidance of hookwire and blue dye, ensuring complete excision of the target SLN(s).

2. All excised nodes were sent for histopathological examination.

3. Results

3.1 Patient characteristics

A total of 76 patients were included in this study. The baseline demographic and clinicopathological characteristics are summarized in Table 1. The mean age of the study population was 51 ± 9 years, and the mean body mass index (BMI) was 23.69 ± 2.79 kg/m². In terms of clinical T stage, 10 patients (13.2%) had Tis disease, 31 (40.8%) had T1 disease, 32 (42.1%) had T2 disease, 2 (2.6%) had T3 disease, and 1 (1.3%) had T4 disease. Regarding tumor location within the breast, 25 tumors (32.9%) were located in the upper outer quadrant, 12 (15.8%) in the upper inner quadrant, 8 (10.5%) in the lower outer quadrant, 4 (5.3%) in the lower inner quadrant, 6 (7.9%) in the central region, and 21 (27.6%) in the overlapping region. Histologic grade 1, 2, and 3 tumors were identified in 28 (36.8%), 44 (57.9%), and 4 patients (5.3%), respectively. Histologically, 10 patients (13.2%) had ductal carcinoma in situ (DCIS), 57 (75.0%) had invasive ductal carcinoma (IDC), 2 (2.6%) had invasive lobular carcinoma (ILC), and 7 (9.2%) had other histological subtypes. With regard to receptor status, 49 patients (64.5%) were estrogen receptor (ER)-positive, 43 (56.6%) were progesterone receptor (PR)-positive, and 28 (36.8%) were HER2-positive.

Table 1
Baseline characteristics of the study population

Characteristic	N = 76 [1]
Age	51 ± 9
BMI	23.69 ± 2.79
Clinical T stage	
1	31 (41%)
2	32 (42%)
3	2 (2.6%)
4	1 (1.3%)
Tis	10 (13%)
Tumor-quadrant	
Central	6 (7.9%)
Lower inner	4 (5.3%)
Lower outer	8 (11%)
Overlapping	21 (28%)
Upper inner	12 (16%)
Upper outer	25 (33%)
Grade	
1	28 (37%)
2	44 (58%)
3	4 (5.3%)
Histology	
DCIS	10 (13%)
IDC	57 (75%)
ILC	2 (2.6%)
Others	7 (9.2%)
ER-status	
-	27 (36%)
+	49 (64%)

Characteristic	N = 76 [1]
PR-status	
-	33 (43%)
+	43 (57%)
HER2 status	
-	48 (63%)
+	28 (37%)
Values are presented as mean ± SD or n (%).	

3.2 Detection performance of CEUS and methylene blue

The detection performance of CEUS and methylene blue staining is summarized in Table 2. Among the 76 patients included in this study, Sentinel lymph nodes were successfully identified in 73 patients using either CEUS or methylene blue, yielding an overall detection rate of 96.05%.

When analyzed separately, CEUS identified SLNs in 71 patients (93.42%), whereas methylene blue staining identified SLNs in 69 patients (90.79%). The detection rate of CEUS was slightly higher than that of methylene blue staining. The use of either CEUS or methylene blue increased the overall detection rate compared with either modality alone, suggesting a complementary role between the two techniques.

Table 2
Detection rates of sentinel lymph nodes by CEUS and methylene blue

Detection method	Patients (n)	Percentage (%)
Either CEUS or methylene blue	73	96.05%
CEUS	71	93.42%
Methylene blue	69	90.79%
Total Patients	76	100%

3.3 Pathological status of sentinel lymph nodes

Pathological examination revealed sentinel lymph node metastasis in 7 of the 76 patients (9.21%). Among these metastatic cases, SLNs were identified by either CEUS or methylene blue staining in 7 patients, including 2 patients detected by CEUS alone, 1 patient detected by methylene blue alone, and 4 patients detected by both methods. These findings suggest that reliance on a single detection modality may lead to missed metastatic SLNs. Some patients had more than one metastatic sentinel lymph node. Therefore, the combined application of CEUS and methylene blue may improve the completeness of SLN detection and reduce the risk of overlooking metastatic nodes.

3.4 Association between CEUS imaging grade and SLN metastasis

A total of 79 sentinel lymph nodes were excised and evaluated. Among them, 8 nodes were confirmed to be metastatic on pathological examination. CEUS imaging grade showed a clear association with the presence of SLN metastasis. Among 55 grade I nodes, 1 node was metastatic, corresponding to a positivity rate of 1.82%. Among 20 grade II nodes, 3 nodes were metastatic, yielding a positivity rate of 15.00%. In contrast, all 4 grade III nodes were metastatic, resulting in a positivity rate of 100%.

These findings indicate that higher CEUS imaging grades were associated with an increased probability of SLN metastasis, suggesting that CEUS imaging characteristics may provide useful information for predicting nodal metastatic involvement.

Table 3
Association between CEUS imaging grade and sentinel lymph node metastasis

CEUS Grade	Total SLNs (n)	Metastatic SLNs (n)	Positivity rate (%)
Grade Ⅰ	55	1	1.82%
Grade Ⅱ	20	3	15.00%
Grade Ⅲ	4	4	100%
ALL	79	8	11.39%

Overall, the probability of SLN metastasis increased progressively with higher CEUS imaging grade, suggesting that CEUS enhancement patterns may provide useful information for predicting nodal metastatic involvement.

4. Discussion

In the present study, we evaluated the clinical performance of contrast-enhanced ultrasound (CEUS)-guided hookwire localization combined with methylene blue staining for sentinel lymph node (SLN) identification and explored the predictive value of CEUS imaging grade for nodal metastasis in patients undergoing SLNB. Three principal findings emerged from this study. First, both CEUS and methylene blue achieved high SLN detection rates, and the use of either CEUS or methylene blue resulted in a high overall identification rate of SLNs. Second, discrepancies were observed between the two detection modalities in metastatic cases, suggesting that reliance on a single technique may lead to missed metastatic nodes, whereas the use of both techniques may improve the completeness of SLN detection by compensating for the limitations of each modality. Third, CEUS imaging grade demonstrated a clear association with pathological SLN metastasis, with markedly higher metastatic rates observed in higher-grade nodes. Taken together, these findings suggest that CEUS-guided hookwire localization combined

with methylene blue staining not only improves the precision of SLN detection but also that CEUS itself may provide useful imaging information for predicting nodal metastatic risk(11–13).

Previous studies have reported encouraging results regarding the application of CEUS for SLN localization in breast cancer. In a prospective study, Wang et al. showed that the combination of CEUS and blue dye achieved a higher identification rate than blue dye alone in clinically node-negative breast cancer patients, supporting the value of multimodal mapping strategies(11). More specifically for localization-guided surgery, Miyake et al. introduced a hookwire-guided CEUS approach (“Sona-Hook”), demonstrating the feasibility of placing a hookwire into the CEUS-identified SLN to facilitate accurate intraoperative retrieval (12). A more recent prospective study by Huang et al. further showed that after preoperative CEUS identification, markers could be deployed into SLNs under ultrasound guidance and then compared with methylene blue tracing intraoperatively, with strong consistency between the two localization methods (13). In addition, Liu et al. reported that preoperative CEUS combined with methylene blue-based nodal tracing represented a feasible minimally invasive SLN tracing strategy in early breast cancer(14). In line with these studies, our findings further support the clinical utility of CEUS-guided hookwire localization as a stable intraoperative landmark, while methylene blue provides complementary visual guidance during surgery.

Another important finding of the present study is that CEUS imaging grade was closely associated with pathological SLN metastasis. This result is consistent with recent evidence indicating that CEUS enhancement patterns are not merely descriptive imaging findings but may reflect the biological status of the node. Zhu et al. demonstrated that CEUS patterns of SLNs were predictive of nodal status and metastatic burden in early breast cancer, suggesting that certain enhancement phenotypes are associated with a higher risk of metastasis (15). Huang et al. subsequently reported that an improved combined percutaneous and intravenous CEUS classification system further enhanced the diagnostic performance for metastatic SLNs (16). More recently, Huang et al. also showed that percutaneous CEUS findings correlated with pathological features of SLNs, further supporting the biological relevance of CEUS-based imaging classification(17). Therefore, our results extend the current evidence by showing that, in the setting of CEUS-guided hookwire localization combined with methylene blue staining, CEUS imaging grade may also serve as a practical indicator for preoperative risk stratification of SLN metastasis.

From a clinical perspective, the present findings suggest that CEUS-guided hookwire localization combined with methylene blue staining may represent a practical and precise strategy for sentinel lymph node biopsy. Conventional dye-guided SLNB relies primarily on intraoperative visualization and surgical experience, which may be influenced by the anatomical variability of lymphatic drainage. In contrast, CEUS allows real-time visualization of lymphatic channels and sentinel nodes before surgery, enabling accurate preoperative localization of the target node. The placement of a hookwire into the CEUS-identified SLN further provides a stable intraoperative landmark, facilitating precise surgical retrieval of the node, while methylene blue offers complementary visual confirmation during surgery. Such an image-guided localization strategy may therefore improve the accuracy and reliability of SLN

identification, particularly in patients with atypical lymphatic drainage patterns or deeper lymph nodes(9, 10).

In addition, the observed association between CEUS imaging grade and SLN metastasis suggests that CEUS may have potential value not only for SLN localization but also for preoperative risk stratification. Previous studies have reported that CEUS enhancement characteristics may reflect microvascular and structural alterations in metastatic lymph nodes, which could help identify nodes with a higher probability of metastasis before surgery(14, 17–19). If validated in larger prospective cohorts, CEUS-based imaging grading systems could potentially assist surgeons in identifying suspicious SLNs preoperatively and optimizing surgical planning.

Overall, the present study demonstrates that CEUS-guided hookwire localization combined with methylene blue staining is a feasible and effective approach for sentinel lymph node identification in breast cancer. The combination of preoperative CEUS localization and intraoperative dye guidance may improve the precision and reliability of SLN detection. In addition, CEUS imaging grade appears to provide useful information for predicting SLN metastasis, suggesting a potential role in preoperative risk stratification. Further large-scale multicenter studies are warranted to validate these findings and to optimize CEUS-based imaging strategies for clinical application.

Abbreviations

SLN
Sentinel lymph node
SLNB
Sentinel lymph node biopsy
CEUS
Contrast-enhanced ultrasound
ALND
Axillary lymph node dissection

Declarations

Author Contributions

The author solely designed the study, collected and analyzed the data, performed the statistical analysis, and wrote the manuscript.

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This research received no external funding.

Conflict of Interest

The author declares that there are no competing interests.

Ethics Approval

This study was approved by the Institutional Review Board of Jiangyou 903 Hospital. Written informed consent was obtained from all participants.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The author declares that there are no competing interests.

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